

C.4.d. Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary Cells

Document #(s): BI Document U87-0467
Upjohn TR 7219-94-081

Conducted by:

Sponsor Volume: 1.44

Summary:

In this clastogenicity study, no reproducible transforming potential of PPX was detected in the Chinese Hamster Ovary cell line. The dosage range used in this study was appropriate, since it approached desired upper treatment levels for cell lines (5 mg/ml or 10 mM = 2.9 mg/ml for PPX) and caused significant cytotoxicity at the highest concentrations. A small, non-reproducible clastogenic effect was observed with activation at the highest test dose (3300 µg/ml).

Methods:

Drug Concentrations

and Exposures: PPX dihydrochloride (Batch I) dissolved in F10/HEPES at the following concentrations:

Without S9 activation: 100, 500, 1000, 2000 µg/ml

With S9 activation: 100, 333, 1000, 3330 µg/ml

Positive Controls/vehicles:

Without S9 activation: Ethylmethanesulfonate (4 or 6 mM) in DMSO

With S9 activation: Cyclophosphamide (10 or 5 µg/ml) in medium

Test System:

Duplicate monolayer cultures of CHO cells (1×10^6) in 75cm² flasks were exposed to test substance for 2 hr. Incubations were continued for hrs. Cell division was arrested at metaphase by addition of colchicine during the last two hrs of the incubation. Chromosomal material was condensed and fixed on a glass slide, stained with 5% Giemsa, and examined microscopically for aberrations (gaps, breaks, fragments, dicentrics, exchange figures, numerical variations). 100 Metaphase spreads per culture were examined by light microscopy.

Metabolizing System:

S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Statistics: Chi-square analysis, $p < 0.05$ (one-tailed test)

Results:

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Determination of cytotoxicity:

Experimental doses were selected based on the following cytotoxicity data:

4.1 Cytotoxicity test/dosage selection

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TABLE 1 CYTOTOXICITY TEST OF SND 919 C12Y

Test substance concentration ($\mu\text{g/ml}$)	Cells/6 cm ϕ culture dish ($\times 10^5$) ^b	
	Directly after exposure (% of control)	After 18-20 h of growth (% of control)
<u>Without metabolic activation (-S9-mix) -</u>		
Control ^a	4.61 (100%)	11.57 (100%)
1	*	*
3.3	4.77 (103%)	12.39 (107%)
10	*	*
33	4.99 (108%)	10.14 (88%)
100	4.38 (95%)	12.08 (104%)
333	2.97 (64%)	10.85 (94%)
1000	3.48 (75%)	11.48 (99%)
3330	1.80 (39%)	3.35 (29%)
5000	0.92 (20%)	0
<u>With metabolic activation (+S9-mix)</u>		
Control ^a	4.41 (100%)	13.25 (100%)
1	*	*
3.3	4.27 (97%)	14.12 (107%)
10	*	*
33	4.57 (104%)	12.05 (91%)
100	4.24 (96%)	*
333	4.35 (99%)	12.20 (92%)
1000	4.57 (104%)	13.40 (101%)
3330	4.48 (102%)	10.17 (77%)
5000	2.71 (61%)	0.50 (4%)

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Cytogenetic Test Results:

In the absence of S9, no increase in the number of chromosomal aberrations was observed in PPX-treated cultures (Table C.4.d.2). However, the positive control (EMS) was only "mildly" clastogenic, so the experiment was repeated. A more robust EMS response was obtained, and PPX appeared clastogenic at a concentration of 500 $\mu\text{g/ml}$ (Table C.4.d.3). Since the effect was rather small and no dose-related trends were observed, it is probably not biologically significant.

In the presence of S9, a significant increase in number of chromosomal aberrations was observed in cultures treated with the high concentration of PPX (3300 $\mu\text{g/ml}$; Tab. C.4.d.4). The effect was relatively small compared to that of cyclophosphamide, and not reproducible (Tab. C.4.d.5).

The frequency of chromosomal aberrations in control cultures were within historical control range for this laboratory (6.6 ± 3.0).

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C.4.d.2.

TABLE 4 CHROMOSOME ABERRATIONS^{a)}: INDIVIDUAL DATA (WITHOUT METABOLIC ACTIVATION; -S9-mix)

Concentration (µg/ml)	Culture	No. of cells scored	No. of cells with aberrations		g	g'	g''	b	b'	b''	f	f'	f''	exch	dic	d'	d''	mfs	Total aberrations (including/excluding gaps)
			included	excluded															
0	A	100	9	5	4	1					9								14/9
	B	100	5	5			2				3								
	Total (A + B)	200	14	10															
100	A	100	7	4	2	1		1			3								7/4
	B	100	5	3	1	1	2				1								5/3
	Total (A + B)	200	12	7															
500	A	100	9	7	2		1				6							sp	10/8
	B	100	9	5	3	1	1				4								9/5
	Total (A + B)	200	18	12															
1000	A	100	3	2	1						2								3/2
	B	100	7	3	2	2	1				2								7/3
	Total (A + B)	200	10	5															
2000	A	100	8	3	5		1				2							poly, endo	8/3
	B	100	9	5	4						3	1						sp, endo	9/5
	Total (A + B)	200	17	8															
4 mM EMS	A	100	15	10	5	1	2	1			8							poly	17/11
	B	100	12	8	3	1	3				5							ma	13/9
	Total (A + B)	200	27*	18															

a) Abbreviations used for various types of aberrations are listed in Appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration. Significantly different from control group: Chi-Square Test, *P < 0.05, **P < 0.01, or ***P < 0.001.

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C.A.d.3

TABLE # CHROMOSOME ABERRATIONS^{a)}: INDIVIDUAL DATA (WITHOUT METABOLIC ACTIVATION; -S9-HIX)

Concentration (µg/ml)	Culture	No. of cells scored	No. of cells with aberrations		g	g	b	b	f	f	exch	dic	misc	Total aberrations (including/excluding gaps)
			included	excluded										
0	A	100	5	3	2	1	1		2					6/3
0	B	100	11	5	9		4		1				2 poly	14/5
	Total (A + B)	200	16	8										
100	A	100	12	9	3		2		6				5D	12/9
100	B	100	7	5	1	1	2		4				3 poly, endo	8/6
	Total (A + B)	200	19	14										
500	A	100	8	8	1		6		1		1		poly	9/8
500	B	100	12	9	3		3		6				2 poly	12/9
	Total (A + B)	200	20	17*										
1000	A	100	8	1	8		1							9/1
1000	B	100	6	2	3	1	2						poly	6/2
	Total (A + B)	200	14	3										
2000	A	100	5	2	3		1				1		3 poly	5/2
2000	B	100	7	2	5		1		1				poly	7/2
	Total (A + B)	200	12	4										
6 mM EMS	A	100	22	16	8	2	10	1	4				sp, poly	26/16
6 mM EMS	B	100	25	20	7		11		11	3			sp	33/26
	Total (A + B)	200	47***	36***										

a) Abbreviations used for various types of aberrations are listed in Appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration. Significantly different from control group: chi-square test, *P<0.05, **P<0.01, or ***P<0.001

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C.4.d.4

TABLE 4 CHROMOSOME ABERRATIONS^a: INDIVIDUAL DATA (WITH METABOLIC ACTIVATION; +S9-mix)

Concentration (µg/ml)	Culture	No. of cells scored	No. of cells with aberrations		'g'	'g''	'b'	'b''	'f'	'f''	exch	dic	'd'	misc	Total aberrations (including/excluding gaps)
			gaps included	gaps excluded											
0	A	100	11	7	3	1	1		6					endo	11/7
0	B	100	6	3	3		2		2						7/4
	Total (A + B)	200	17	10											
100	A	100	10	5	4	1			4			1			10/5
100	B	100	11	8	4	1	3		4				ma		13/8
	Total (A + B)	200	21	13											
333	A	100	2	1	1				1						2/1
333	B	100	12	7	5		2		4			1	endo		12/7
	Total (A + B)	200	14	8											
1000	A	100	12	10	3		1		7			2	sp		14/11
1000	B	100	8	6	2		1	2	4						9/7
	Total (A + B)	200	20	16											
3330	A	100	19	15	4		3		13					3ma, poly, endo	23/19
3330	B	100	15	12	5		2		4		2			6ma, 3poly, endo, 2sp	21/16
	Total (A + B)	200	34**	27**											
10 µg/ml CP	A	50	42	35	7	1	13		25		20			2ma, endo, 2 sp	70/62
10 µg/ml CP	B	50	40	38	9	4	10		29		10			SP, 6ma	69/56
	Total (A + B)	100	82***	73***											

a) Abbreviations used for various types of aberrations are listed in Appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration. Significantly different from control group: Chi-Square Test, * P<0.05, **P<0.01, or ***P<0.001

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C.A.d.5

TABLE 0. CHROMOSOME ABERRATIONS^a: INDIVIDUAL DATA (WITH METABOLIC ACTIVATION; +S9-mix)

Concentration (µg/ml)	Culture	No. of cells scored	No. of cells with aberrations		g	b	b	f	f	exch	dic	misc	Total aberrations (including/excluding gaps)
			included	gaps excluded									
0	A	100	11	9	3	1	3	1	5			poly, 2 endo	13/9
	B	100	5	4	2		1		3			2 poly, 4 endo	6/4
	Total (A+B)	200	16	13									
333	A	100	9	3	6		2		1				9/3
	B	100	14	7	6	3	2		5			2 endo, 1 poly	16/7
	Total (A+B)	200	23	10					6			endo, poly, r	17/12
1000	A	100	14	11	4	1	5		2			2 endo, poly	10/6
	B	100	9	6	4		4		2				
	Total (A+B)	200	23	17								4 endo, poly	11/6
2500	A	100	10	6	5		4		1		1		
	B	100	11	7	5		4		3			2 poly	12/7
	Total (A+B)	200	21	13									
3330	A	100	13	10	4		6		4			1 endo	14/10
	B	100	9	5	5		3		3			poly, endo	11/6
	Total (A+B)	200	22	15									
5 µg/ml CP	A	50	45	44	7	3	21		14	16	2	14 ma, 4 sp	81/71
	B	50	49	49	8	16		41	23			10 ma, 1 sp	101/93
	Total (A+B)	100	94	93	15	37		55	39				

a) Abbreviations used for various types of aberrations are listed in Appendix 1. The numerical variations endoreduplication (endo) and polyploidy (poly) were not counted as an aberration. Significantly different from control group: chi-square test, *P < 0.05, **P < 0.01, or ***P < 0.001

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C.4.e. V79 Gene Mutation Assay for HGPRT Mutants

Document #(s): BI Document U90-0385
Upjohn TR 7219-94-083

Sponsor Volume: 1.44

Summary:

In this mutagenicity study, PPX did not produce mutations in the HGPRT locus in the Chinese Hamster V79 cell line. The dosage range used in this study was appropriate, since it approached desired upper treatment levels for cell lines (10 mM pramipexole = 2.9 mg/ml for pramipexole). The survival rate at the high concentration of 3000 µg/ml was Positive controls produced the expected mutations.

Methods:

Drug Concentrations and Exposures: PPX dihydrochloride (Batch III) dissolved in DMEM as follows:

Expt. I:

Without S9 activation: 100, 500, 1000, 2500, 3000 µg/ml

With S9 activation: 100, 1000, 2000, 3000 µg/ml

Expt. II:

Without S9 activation: 100, 1000, 2500, 3000 µg/ml

With S9 activation: 100, 1000, 2000, 3000 µg/ml

Positive Controls/vehicles:

Without S9 activation: Ethylmethanesulfonate (500 µg/ml) dissolved in treatment medium

With S9 activation: DMBA (8 µg/ml) in DMSO (final conc = 1% in medium)

Test System:

The basis of this study is the presence/absence of hypoxanthine-guanine phosphoribosyl transferase in V79 cells. A mutation in the HGPRT locus results in cells which do not convert 6-thioguanine (6-TG) into a toxic metabolite, and thus survive treatment with media containing 6-TG. Mutants arise from base-pair substitutions, frameshifts, deletions, and chromosome rearrangements.

V79 Chinese hamster cells (5×10^5) were cultured in 80 cm² flasks for mutation

studies (1 flask per point). For survival analysis, 220 cells were plated in 58 cm² dishes in triplicate. Cells were exposed to test substance, with and without S9 fraction, for 4 hr. Cells in the survival study were fixed and stained on day 6. Cells in the mutation study were subcultured every 2-3 days. On day 7, cells (5x10⁵) were plated into 58 cm² dishes and medium containing 6-TG was added to cultures for mutant selection. An additional set of 58 cm² dishes were seeded with 220 cells to which complete medium was added for determination of plating efficiency. Incubations were continued with media changes for 6-7 days. Cultures were fixed (ethanol/glacial acetic acid, 3:1) and stained (7% Giemsa) on days 13 and 14 after culture initiation for determination of plating efficiency and mutant selection, respectively. Colonies containing more than 50 cells were counted.

Metabolizing System: S9 fraction prepared from Arochlor-induced rat (500 mg/kg given five days before tissue harvest)

Results:

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Determination of cytotoxicity:

Significant cytotoxicity occurred with concentrations of 5000 µg/ml in the presence or absence of S9 (Tab. C.4.e.1). Thus, 3000 µg/ml was chosen as the high dose for mutagenicity testing.

Table 1: Results of the preliminary experiment on toxicity

(Experimental parts no. MUT 0127/01 and /02)

Substance	Concentration* (µg/ml)	Number of colonies per plate		Survival %
		Individual counts	Mean	
<u>Without S9 mix:</u>				
Negative control		222, 249, 233	235	100
SND 919 CL2Y	10	227, 234, 231	231	98
	100	238, 207, 216	220	94
	1000	106, 127, 105	113	48
	2500	111, 89, 92	97	41
	5000	25, 23, 17	22	9
<u>With S9 mix:</u>				
Negative control		244, 256, 238	246	100
SND 919 CL2Y	10	235, 240, 229	235	96
	100	252, 228, 239	240	98
	1000	230, 248, 249	242	98
	2500	245, 227, 201	224	91
	5000	81, 86, 53	73	30

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Mutagenicity Test Results:

The mutation rate in cultures treated with PPX was approximately equivalent to negative control rates in the presence or absence of S9 in both experiments. The mutation frequency in these cultures were within the normal range of this laboratory. The positive controls EMS and DMBA produced the expected significant increases in mutation rate frequency (Tables C.4.e.2-3). Similar results were obtained in a replicate experiment.

C.4.e.2.

Table 2: Mutagenicity assay; experiment 1; without S9 mix

(Experimental part no. MUT 0127/03)

Toxicity data

Substance	Concentration ^a (µg/ml)	Number of colonies per plate		Survival %
		Individual counts	Mean	
Negative control		215, 218, 201	211	100
SND 919 CL2Y	100	206, 188, 209	201	95
	500	186, 206, 208	200	95
	1000	199, 188, 177	188	89
	2500	131, 123, 160	138	65
	3000	118, 110, 131	120	57
Positive control with EMS	500	183, 176, 190	183	87

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Mutation induction

Substance	Conc. ^a (µg/ml)	Plating efficiency		Number of cells seeded per flask (B)	Mutant selection ^b		Mutants per 10 ⁶ survivors (D)
		Individual plate counts	Mean (A) ^c		Individual plate counts	Mean (C)	
Negative control		77, 109, 101	96	238806	0, 0, 3	1.0	4.2
SND 919 CL2Y	100	80, 77, 85	81	199507	0, 0, 0	0	0
	500	103, 117, 104	108	259615	1, 6, 1	2.7	10.4
	1000	61, 77, 85	74	184080	0, 1, 0	0.3	1.6
	2500	120, 117, 103	113	278325	0, 0, 1	0.3	1.1
	3000	126, 112, 110	116	287129	0, 0, 0	0	0
Positive control with EMS	500	72, 79, 68	73	180693	129, 144, 123	132.0	730.5

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For footnotes and abbreviations see Table 1.

^a Based on number of seeded cells: neg. control, 201; 100 µg/ml, 203; 500 µg/ml, 208; 1000 µg/ml, 201; 2500 µg/ml, 203; 3000 µg/ml, 202; pos. control, 202.

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C.A.e.3.
Table 2: Mutagenicity assay; experiment 1; with S9 mix

(Experimental part no. MUT 0127/05)

Toxicity data

Substance	Concentration* (µg/ml)	Number of colonies per plate		Survival %
		Individual counts	Mean	
Negative control		210, 213, 187	203	100
SND 919 CL2Y	100	202, 176, 184	187	92
	1000	174, 145, 178	166	82
	2000	178, 189, 169	179	88
	3000	125, 153, 131	136	67
Positive control with DMBA	8	74, 65, 64	68	33

Mutation induction

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Substance	Conc.* (µg/ml)	Plating efficiency		Number of cells seeded per flask (B)	Mutant selection ^b		Mutants per 10 ⁶ survivors (D)
		Individual plate counts	Mean (A)		Individual plate counts	Mean (C)	
Negative control		167, 165, 179	170	425000	7, 7, 5	6.3	14.8
SND 919 CL2Y	100	174, 171, 176	174	435000	0, 1, 1	0.7	1.6
	1000	173, 182, 190	182	455000	2, 1, 1	1.3	2.9
	2000	175, 180, 179	178	445000	3, 2, 1	2.0	4.5
	3000	194, 186, 187	189	472500	0, 0, 1	0.3	0.6
Positive control with DMBA	8	92, 94, 123	103	257500	250, 295, 221	255.3	991.5

For footnotes and abbreviations see Table 1.

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C.4.f. *In vivo* Mouse Micronucleus Test

Document #(s): BI Document U85-0651
Upjohn TR 7219-94-080

Sponsor Volume: 1.44

Summary:

In this *in vivo* mutagenicity study, a single, high, toxic dose of PPX (1000 mg/kg) did not significantly increase the number of micronucleated polychromatic erythrocytes in the femoral bone marrow of mice at _____ hrs after dosing. The positive control cyclophosphamide produced the expected result.

According to 1994 OECD guidelines, this study is deficient with respect to the number of doses employed (one rather than the recommended three), and the number of polychromatic erythrocytes scored for the presence of micronuclei (1000 vs recommended 2000). Since there was no indication that a single high toxic dose of PPX causes even the slightest increase in the occurrence of micronuclei, and a high rate of lethality would be expected at the recommended 2000 mg/kg dose level, a repetition of this study to conform with guidelines will not be required.

Methods:

Dosage/Route: 1000 mg/kg PPX dihydrochloride (Batch C in 10 ml/kg distilled water) by gavage.

Positive Controls/vehicles: Cyclophosphamide

Animals: Chbb:NMRI mice, 5 g, 10 weeks old.
35 animals (17 M, 18 F) received drug; 2 M and 3 F died within 1 hr of dosing.

Dosing Regimen:

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Group	Dose	Sampling time	Sex	Animal number
0	dist. water	24 h	male female	001 - 005 051 - 055
1	SND 919 CL 2 1000 mg/kg	24 h	male female	101 - 106 151 - 156
2	SND 919 CL 2 1000 mg/kg	48 h	male female	201 - 206 251 - 256
3	SND 919 CL 2 1000 mg/kg	72 h	male female	301 - 305 351 - 356
4	Cyclophosphamide 50 mg/kg	24 h	male female	401 - 405 451 - 455

Note: Failure to comply with OECD guidelines stating that 3 dose levels, or a single dose level of 2000 mg/kg/day should be employed.

Sample Analysis: Femoral marrow smears were fixed on a slide (2 per animal) and stained with Giemsa. At least 1000 polychromatic erythrocytes per animal were scored for the presence of micronuclei. The ratio of polychromatic to normochromatic nuclei was determined by counting 1000 erythrocytes.

Note: OECD guidelines state that 2000 PCEs should be scored.

Results:

A single dose of 1000 mg/kg PPX to male and female mice did not significantly increase the number of micro nucleated polychromatic erythrocytes relative to control levels at 24, 48 or 72 hrs. The ratio of polychromatic to normochromatic erythrocytes also was not affected by PPX. The positive control cyclophosphamide produced the expected increase micronucleated polychromatic erythrocytes.

Table 1: Results of Micronucleus Test with SND 919 CL 2

Dose ^a	Sampling time	Sex	Number of mice	Micronucleated polychromatic erythrocytes (‰) Mean ± standard deviation
Vehicle control	24 h	male	5	1.8 ± 0.4
		female	5	1.2 ± 0.8
SND 919 CL 2 1000 mg/kg	24 h	male	5	2.6 ± 1.3
		female	5	2.2 ± 1.3
SND 919 CL 2 1000 mg/kg	48 h	male	5	1.6 ± 0.9
		female	5	2.2 ± 0.8
SND 919 CL 2 1000 mg/kg	72 h	male	5	2.2 ± 0.8
		female	5	1.2 ± 0.4
Cyclophosphamide 50 mg/kg	24 h	male	5	31.4 ± 8.1 ^b
		female	5	23.0 ± 5.4 ^b

^a Vehicle control, 10 ml dist. water per kg.

^b Significantly different from the vehicle control ($\alpha \leq 0.01$).

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C.5. Carcinogenicity

C.5.a. Two-Year Mouse Carcinogenicity Study

Conducted by : Boehringer Ingelheim KG
Depts. of Experimental Pathology and Toxicology,
and Pharmacokinetics and Metabolism
Research and Development Coordination
Muttentz, Switzerland

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Document #(s): BI Document U93-0589
Upjohn TR 7219-94-070

Sponsor Volumes: 1.45-1.48

This study complied with GLP

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Summary:

Pramipexole was administered in the diet at doses of 0.3, 2.0, and 10.0 mg/kg/day to Chbb:NMRI mice (50/sex/dose group, 100/sex/control) for two years. Relatively few non-neoplastic and no neoplastic lesions were clearly associated with PPX administration.

The rate of premature decedents was higher in PPX-treated animals than in controls; the effect was significant in males. The highest mortality rate was 46% in MDF and HDF. The primary cause of premature deaths were unscheduled sacrifices due to eczema, a condition observed in both control and treated animals. Body weight gain was significantly reduced by % in both sexes at the intermediate and high doses at study termination. A relative increase in the incidence of alopecia was also noted in PPX-treated animals. Spontaneous activity was increased in MD and HD females, and HD males.

No statistically significant increases or trends for increases in the incidence of neoplastic lesions in drug-treated animals were apparent according to the sponsor's analysis. A pooled analysis of all mesenchymal/epithelial uterine neoplasms was not presented, but the incidences suggest a possible dose-related positive trend (controls: 10%; LD: 10%; MD: 14%; HD: 16%). Statistically significant decreases in the incidence of adrenal cortical adenomas in HD males, and malignant lymphomas in MD and HD females were noted. For all other neoplastic findings, which included systemic neoplasms of the hemolymphoreticular system, and primary neoplasms in the lung, liver, and adrenals in males, and the reproductive tracts of both sexes, the incidences were low, and equivalent in PPX-treated and control animals.

The only histopathological findings that occurred at a higher incidence rate in PPX-treated animals were fibro-osseous proliferative lesions in the femurs of females (all dosage groups). This lesion occurred at a relatively high rate in control females (28%), but approximately doubled in incidence in treated animals %; similar at the three dosage levels). The more severe lesions were found more frequently in treated animals. This type of lesion is known to occur spontaneously in female mice of other strains including B6C3F1 (Albassam, *et al.*, Vet. Pathol., 28:381, 1991), and has also been observed in mice after

administration of the prostaglandin E analogue misoprostol (Dodd and Port, Vet. Pathol., 24:545, 1987) and estrogens (Gaunt and Pierce, Vet. Pathol., 22:403, 1985). The increased incidence in drug-treated animals may be related to stimulation of estrogen release (Sass and Montali, Lab. Anim. Sci., 30:907, 1980), although no experimental evidence of such a hormonal effect of PPX was presented. Pathological changes that might be expected to accompany a bone abnormality (i.e., blood cell count changes) were not clearly associated with this lesion. Possibly compensatory stimulation of splenic erythropoiesis occurred in both treated and control female mice, and increased hematopoietic activity was noted in the femoral bone marrow of MDF and HDF.

Based on plasma level measurements in satellite groups during weeks 2, 40 and 80 at 4-5 hrs after light onset, exposure to PPX in the high dose group (ng/ml) was fold higher than the C_{max} in humans following the expected maintenance dose of 1.5 mg, t.i.d.

Thus, administration of PPX in the diet for two years was not significantly carcinogenic in mice. However, conclusive interpretation of these results is hindered by the marked impairment of body weight development at the mid- and high-dose levels. The low exposures at the lowest dose levels cannot be considered adequate for assessing the tumorigenic effects of this compound. The importance of the fibro-osseous proliferative lesion is questionable since similar lesions are known to occur spontaneously in certain strains of mice, and no similar lesion was observed in long-term rat and monkey PPX studies. The "No Effect" dose was considered to be 0.3 mg/kg/day, although a trend for decreased food intake was apparent at this dose.

Methods:

Dosages: 0.3, 2.0, 10.0 mg/kg/day PPX dihydrochloride (Batch II)

The low dose is three times the ED₅₀ for anti-Parkinsonian effects in monkeys, and 5-15 times higher than the expected human maintenance dose range of 1.5-4.5 mg/day (70 kg human). The high dose was selected as the highest tolerable dose given the duration of the study and the limitation of excessive CNS stimulation. The reduction in body weight gain by this dose was used as an indicator of drug toxicity.

Route of Administration: Drug-in-diet

Species/Strain/Number: Mouse (Chbb:NMRI)

250 males, 250 females for toxicology
20 males, 20 females for microbiology
159 males, 159 females for toxicokinetics

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Toxicology Groups:

Group size and dosage:

Group	Dbse mg/kg	Number of animals		Identity number
		males	females	
0 (control A)	0	50	50	0001-0050 0501-0550
1	0.3	50	50	1001-1050 1501-1550
2	2.0	50	50	2001-2050 2501-2550
3	10.0	50	50	3001-3050 3501-3550
4 (control B)	0	50	50	4001-4050 4501-4550

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Toxicokinetic Analyses:

Group no.	Animals/sex per sampling	Animals/ plasma pool	Pooled samples/ group & date
5	20 / m	5	4
	20 / f	5	4
6	16 / m	4	4
	16 / f	4	4
7	12 / m	3	4
	12 / f	3	4

Blood was sampled during weeks 2, 40 and 80 at 10.00 to 11.00 AM (4-5 hrs after light onset).

Mean initial weights/age:

males: 29.3g / 37 days
females: 24.5g / 37 days

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Parameters monitored/Intervals:

Clinical - daily
Body weight - weekly (wks 1-26), monthly (wks 27-104)
Food consumption - weekly
Water consumption - weekly (weeks 14, 26, 39, 52, 65, 78, 91, 104)
Effective dose - calculated weekly (wks 1-26); monthly thereafter
Hematology - done only prior to sacrifice
Plasma Conc - in satellite groups as described above
Histopathology - on the following tissues:

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Adrenal glands	Rectum
Aorta	Salivary glands
Brain	Seminal vesicle
Caecum	Skeletal muscle
Cervical lymph node	Skin
Colon	Spinal cord
Duodenum	Spleen
Femur/stifle joint (incl. bone marrow)	Sternum ¹⁾
Heart	Stomach
Ileum	Testes with epididymides ²⁾
Jejunum	Thymus
Kidneys	Thyroid gland
Larynx ¹⁾	Tongue
Liver/gallbladder	Trachea
Lungs	Urinary bladder
Mammary gland area	Uterus with cervix
Mesenteric lymph node	Vagina
Oesophagus	
Ovaries	Both pinnae with ear tattoo ¹⁾
Pancreas	
Parathyroid glands	All gross lesions incl. tumours/suspected tumours and regional lymph nodes
Periph. (sciatic) nerve	
Pituitary gland	
Prostate gland	

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Both eyes with optic nerve and Harderian glands were fixed in Heidenhein's Susa solution.

1)= conserved but not prepared histologically.
2)= fixed in Bouin's solution.

Stains:	Hematoxylin/Eosin -	all organs/tissues, tumors/lesions
	Masson's Trichrome -	heart, kidney, liver, gall bladder lung, aorta, tumors/lesions

Statistics

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Routine group comparisons were made by the Bartlett test, one-way ANOVA and Newman-Keuls test. The Exact Log-rank test was used for group comparisons of categorical tumor-bearing animal data, and for between-group comparisons of the number of premature decedents.

Plasma concentration data were evaluated after logarithmic transformation by regression analysis and ANOVA to determine the effects dose, time point and sex.

Statistical evaluation of neoplastic lesions was according to Peto et al. (1980) using the trend test with respect to dose. Probability levels for significant findings were 0.05 for rare neoplasms and 0.01 for common neoplasms.

Results:

Mortality: 87 males and 101 females died or were sacrificed moribund prior to the end of the study.

Group Sex	Contr. A		Contr. B		1		2		3	
	m	f	m	f	m	f	m	f	m	f
Died	7	4	3	5	8	10	9	8	15	5
Sacr.	6	9	7	15	13	13	13	14	6	18
Total	13	13	10	20	21	23	22	22	21	23
†	26	26	20	40	42	46	44	44	42	46

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The increased mortality in treated males was statistically significant ($p = 0.0298$ by a one-tailed positive trend test; $p = 0.0112$ by heterogeneity test). The major factor contributing to the higher mortality rate was sacrifice due to debilitating eczema.

Skin lesions, in particular eczematous changes and frank dermal ulceration, were a significant reason for sacrificing animals during study. As is evident from the following table, the incidence of these lesions in premature decedents was somewhat higher in the treated groups:

Controls		Group 1		Group 2		Group 3	
m	f	m	f	m	f	m	f
Absolute values:							
5	3	6	0	8	2	5	5
As a percentage of total animals in group:							
5	3	12	0	16	4	10	10

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Causes of death are listed in Table C.5.a.1

Table C.5.a. Causes of Death or Sacrifice:

Controls (A&B)			
<u>Males</u>	<u>23 deaths</u>	<u>Females</u>	<u>33 deaths</u>
neoplasia	- 10	neoplasia	- 22
dermatitis/eczema	- 4	eczema	- 1
edema	- 3	hemometra	- 1
botryomycosis	- 2	wound	- 1
undetermined	- 4	atrial thrombosis	- 1
		posterior paralysis	- 1
		amyloidosis	- 1
		undetermined	- 5
Low-Dose			
<u>Males</u>	<u>21 deaths</u>	<u>Females</u>	<u>23 deaths</u>
neoplasia	- 9	neoplasia	- 13
eczema	- 3	amyloidosis	- 3
edema	- 1	hemometra	- 1
botryomycosis	- 1	glomerulosclerosis	- 1
ulceration	- 1	skull fracture	- 1
glomerulosclerosis	- 1	undetermined	- 4
undetermined	- 5		
Mid-Dose			
<u>Males</u>	<u>22 deaths</u>	<u>Females</u>	<u>22 deaths</u>
neoplasia	- 4	neoplasia	- 9
dermatitis/eczema	- 8	amyloidosis	- 1
edema	- 3	eczema	- 2
purulent prostatitis	- 1	hemorrhage	- 2
brain hemorrhage	- 1	hemorrhagic cyst	- 1
undetermined	- 5	pyometra	- 1
		peritonitis	- 1
		abscess	- 1
		circulatory failure	- 1
		undetermined	- 3
High-Dose			
<u>Males</u>	<u>21 deaths</u>	<u>Females</u>	<u>23 deaths</u>
neoplasia	- 4	neoplasia	- 12
eczema	- 3	dermatitis/eczema	- 2
edema	- 3	wound	- 1
abd. wall perforation	- 1	botryomycosis	- 1
pyelonephritis	- 1	skin erosion	- 1
skin erosion	- 1	circulatory failure	- 1
mucosal hemorrhage	- 1	caudal paralysis	- 1
undetermined	- 7	pyometra	- 1
		undetermined	- 3

Clinical Signs:

Observation	Contr. A+B	Group		
		1	2	3
Incr. spont. activity	0	0	0	96
Alopecia	12	14	18	42

Males

Observation	Contr. A+B	Group		
		1	2	3
Incr. spont. activity	0	0	98	98
Alopecia	19	20	52	52
Skin lesion	6	6	18	20

Females

Body Weight Gain (Fig. C.5.a.1):

- M & HDM - sig. decrease - all time points
- LDF - sig. increase - wks 1-3, 5-6, 9, 15, 17, 19-70, 78, 86-98
- M & HDF - sig. decrease - from wk 3 to end of study

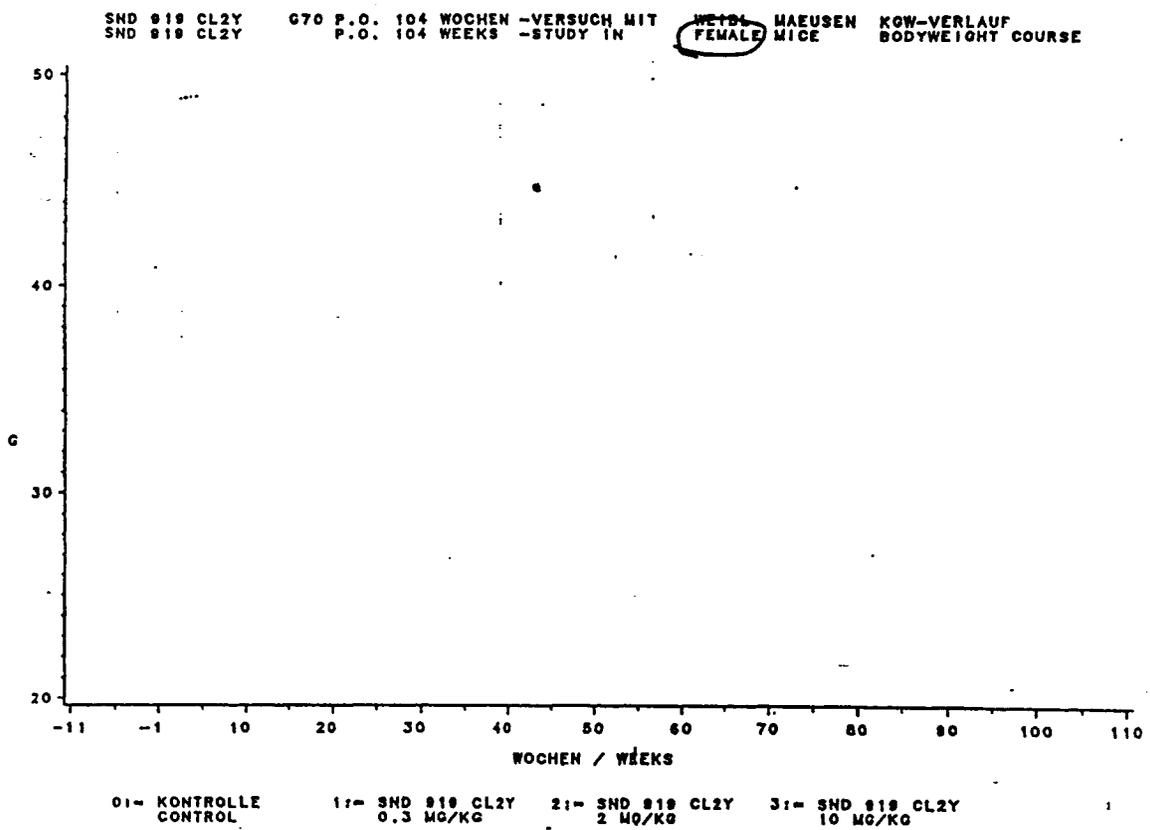
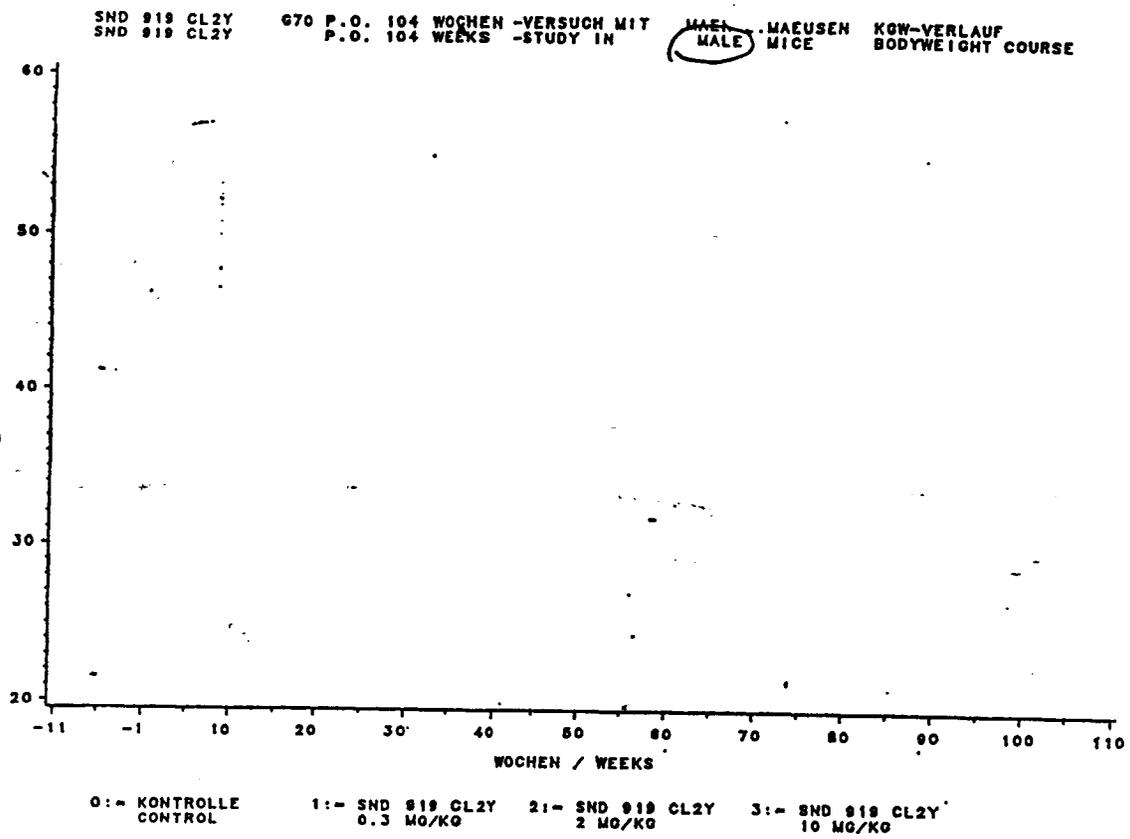
Food Intake:

- LDM - tendency for decrease; effect was significant at several time points
- M & HDM - tendency for increase; effect was significant at several time points
- LDF - tendency for decrease at wks 25-78
- M & HDF - tendency for increase; effect was significant at several time points

Water Intake:

- LDM - no effect
- M & HDM - tendency for increase; effect was significant at several time points
- LDF - no effect
- M & HDF - tendency for increase; effect was significant at several time points

Fig. C.5.a.1.



Alopecia:

	<u>Male</u>	<u>Female</u>
CON	12%	19%
LD	14%	20%
MD	18%	52%
HD	42%	52%

Effective dose: Weekly recordings indicated that effective drug intake was usually within 20% of intended intake. Most variations were in the direction of "greater than intended" intakes.

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	1	Group 2	3
Intended dose (mg/kg)	0.3	2.0	10.0
Males			
Effective dose (mg/kg)			
Mean	0.31	2.11	10.19
Range			
Percentage of intended dose			
Mean	102	105	102
Range			
Females			
Effective dose (mg/kg)			
Mean	0.31	2.11	10.07
Range			
Percentage of intended dose			
Mean	103	105	101
Range			

Hematology:

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A number of animals had abnormal WBC counts at sacrifice. This included several controls as well as PPX-treated animals, and there was no clear dose-relationship. (The sponsor has not indicated their criteria for the noted hematological findings in individual animals).

WBC

Individual variations in animals with abnormal blood counts at termination (Tab. C.5.a.2):

Tab. I. Control animals sacrificed at the end of the study

animal no.	leucocytosis	relative		blasts	anaemia
		lymphocytosis	granulocytosis		
0018	+++	+++			
0020	+	+++			+
0040	.				
0045	+	++			+
0501	+	+++			
0534	+	++			
4008	.	++			
4025	.	++			
4041	.	++			
4044	.				
4045	++	++			
4049	+	++			
4519	++	++			
4548	.	++			

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Tab. II Treated animals sacrificed at the end of the study

animal no.	leucocytosis	relative		blasts	anaemia
		lymphocytosis	granulocytosis		
1014	.	.			
1043	.	+++			.
2006	.		++		
2027	+++	+++			
2031	+	++			
3037	.	.			
3517	leucopenia				
3519	+++	+++			
3535	.	.			

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In addition, the following hematological changes were noted in animals with "normal" WBC counts:

- anemia, slight - 2 OM, 3 OF
3 LDF
2 MDM, 1 MDF
- " , moderate - 3 HDF
- " , marked - 1 OM
1 LDF
1 OF
- erythrocytosis - 1 OM, 1 OF
1 LDF
1 HDM

WBC

Individual variations in animals with abnormal blood counts sacrificed moribund (Tab. C.5.a.3)

Tab. III Control animals sacrificed moribund

animal no.	leucocytosis	relative		blasts	anaemia
		lymphocytosis	granulocytosis		
0029	•		••		
0510	••				•
0527	•				••
0540	••		••		
4026	•				
4036	•				
4527	•				•

sarcoma, body ca
lymphoma, thymic
" non-thymic
leiomyxoma
botryomycosis
" "
paralysis

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Tab. IV. Treated animals sacrificed moribund

animal no.	leucocytosis	relative		blasts	anaemia
		lymphocytosis	granulocytosis		
1037	•		•		*
1039	•		••		
1040	•		•		
1041	•				
1046	•		•		
1506	•		•• 1)		•
1517	•••	•••			•
1537	•				•
2011	•		•		•••
2022	•••	•••			
2039	•				
2047	•		1)	•	
2505	•		•• 1)		•
2507	•	••			••
2512	•	•			
3048	•		1)		•
3502	•••		• 1)		
3518	••		••		•••
3522	•				••
3532	•				••
3538	••	•••			

Sacrifice
Cause of Death
histio. sarc
?
histio. sarc
eczema
histio. sarc
thymic lymphoma
non-thy "
squamous CA (skin)
sarcoma
thymic lymphoma
eczema
"
peritonitis
histio. sarc
?
eczema
botryomycosis
stromal cell CA (uto
sarcoma
eczema
thymic lymphoma

1) shift to the left

* polycythemia

The following hematological changes were noted in animals with "normal" WBC counts:

anemia, slight	-	1 OM, 3 OF 1 LDM, 5 LDF 3 MDM, 5 MDF 3 HDM, 6 HDF
" , moderate	-	6 OF 1 LDF 2 MDF 3 HDF
" , marked	-	1 HDF (this animal was also stated to have polycythemia???)

Group variations:

Statistically significant mean changes were noted on various parameters, but few clearly dose/drug-related effects were evident.

At termination:

increased Hct	-	HDM
decreased lymphocytes(%)	-	MDM, HDM
increased lymphocytes(%)	-	LDF

Moribund sacrifices:

decreased RBC, Hb	-	trend (N.S.) in males
decreased Hct	-	HDM
increased leucocytes	-	MDM (2° to 1 abnormally high value)
increased lymphocytes(%)	-	MDM

(The page containing mean values of RBC parameters for females sacrificed moribund was omitted.)

Plasma Concentrations:

The concentration of PPX was above the LOQ (0.1 ng/ml) in all samples at 4-5 hrs after light onset. Increases in plasma concentrations were approximately dose-proportional except for females during week 2 and both sexes during week 40 where the increases were greater than dose proportional (Fig. C.5.a.2; Tab. C.5.a.4). ANOVA indicated that significantly higher concentrations were present in females, although specific occurrences of this finding were not indicated (Tab. C.5.a.5). There was no evidence of drug accumulation.

Tab.
C.5.a.4.

Sex	Dose [mg/kg]	Week		
		2	40	80
male	0.3	1.28	1.19*	1.21
male	2.0	12.48	3.96 ✓<	7.48
male	10	41.27	69.69 ✓>	36.02
female	0.3	0.99	0.92*	1.77
female	2	10.55	9.09	9.54
female	10	79.81 ✓>	80.80 ✓>	53.14

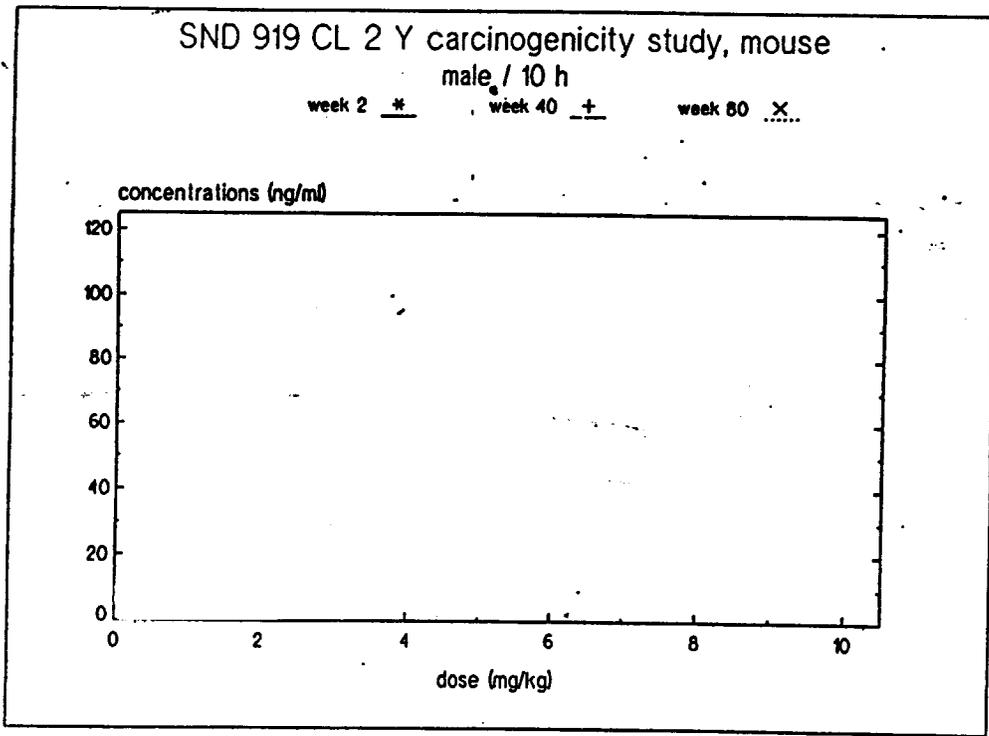
SND 919 C12 Y / carcinogenicity study / mouse
analysis of variance of the log-transformed plasma concentrations

Tab.
C.5.a.5

variability	df	mean squares	p value
between mean values of			
- weeks	2	0.07801	0.068
- sexes within weeks	3	0.09219	0.026
common regression	1	32.80579	< 0.001
between slopes of			
- weeks	2	0.13793	0.010
- sexes within weeks	3	0.05935	0.104
about regression lines	6	0.09966	0.004
between pooled samples.	52	0.02753	
total	69		

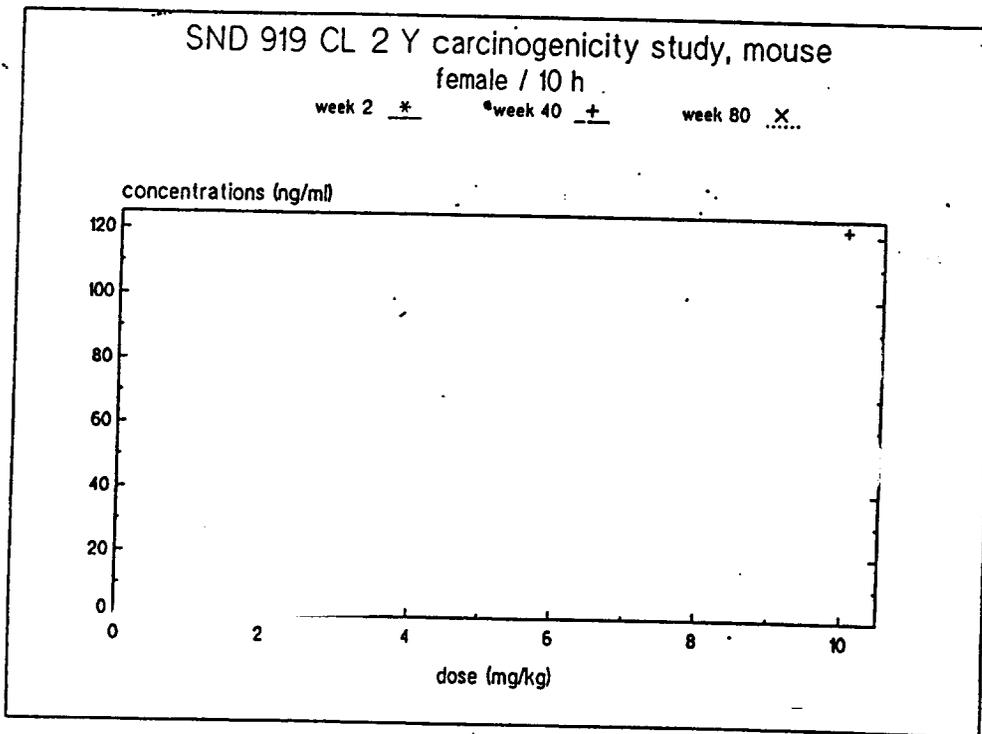
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Fig. C.5.a.2



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Pathology:

Non-Neoplastic Lesions

Statistically significant drug effects were:

Lesion	Group Incidence Rate (%)				
	0	4	1	2	3
Fibro-osseous proliferative lesion (femur) - increase (F)	36	20	62*	60*	56*
Fat marrow (femur epiphysis) - decrease (F)	48	56	42	20*	21*
seminiferous tubule atrophy - decrease	36	36	18	18*	14*

The most notable finding was the fibro-osseous proliferative lesion in the femurs of female mice, which was not described in detail by the sponsor, nor was any potential significance suggested. The lesion occurred spontaneously in control animals, but its incidence was significantly increased by PPX treatment; the incidence rates were similar in all dosage groups. A possibly related finding was decreased femoral fat content in MDF and HDF, suggestive of increased hematopoietic activity.

Albassam et al. (Vet. Pathol., 28:381, 1991) have reported upon the spontaneous occurrence of an apparently similar osseous lesion in the femurs and sternums of female B6C3F1 mice (but not male B6C3F1 or female CF1 mice). The lesion was characterized by the lining of epiphyseal plates by large osteoblasts and had large vascularized centers. Similar drug-induced lesions have been produced by estrogens (Silberberg and Silberberg, Gerontology, 16:201, 1970; Gaunt and Pierce, Vet. Pathol., 22:403, 1985), and the sponsor speculates that a dopaminergic-induced imbalance in estrogen:progesterone levels may account for this lesion. No experimental support for this mechanism (i.e., estrogen level measurements) was provided. The prostaglandin E analogue misoprostol also produces an osseous lesion in female mice (Dodd and Port, Vet. Pathol. 24:545, 1987), and the finding appears in the labeling of that product.

Neoplastic lesions

According to the sponsor's analysis, the only statistically significant differences in the incidence of neoplasia between treated and control animals were decreased occurrences of the following tumors:

Neoplasm	Group/Incidence rate (%)				
	0	4	1	2	3
malignant lymphomas - decrease (F)	46	42	32	22*	16*
adrenal cortical adenomas - decrease (M)	32	16	16	12	6*

There was also a non-significant trend for decreased occurrence of hepatocellular adenomas in treated males.

Inspection of the Tumor Distribution Summary (Tab. C.5.a.6) and statistical analysis tables (Tab. C.5.a.7) reveals some notable findings or tendencies. The incidence of uterine stromal polyps tended to increase at the higher doses (controls: 2%; LD: 2%; MD: 6%; HD: 6%; $p = 0.0778$ by the Trend Test), as did the incidence of all mesenchymal/epithelial uterine neoplasms (control: 10%; LD: 10%; MD: 14%; HD: 18%; not statistically analyzed). The Test of Heterogeneity indicated a statistically significant increase in the incidence of histiocytic sarcomas in PPX-treated male mice ($p = 0.0018$), but the tumors were found only in 4 LD animals and are thus not clearly drug-related.

There were no significant differences between PPX-treated and control animals with respect to the number of primary neoplasms, the number of mice with primary neoplasms, mice with more than one neoplasm, mice with metastases, the number of benign and malignant neoplasms per group and sex (Tab. C.5.a.8).

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TR No.: 7219-94-070

Tab. C.5.a.6

BOEHRINGER INGELHEIM KG Name of finished product		TABULATED STUDY REPORT				U93-0589								
Name of active ingredient Pramipexole (SND 919 CL 2 Y)		ref. to III.E.210												
		2/6				Page Number								
ONCOGENIC/CARCINOGENIC POTENTIAL Tumour data														
Ref. to document.: Volume: Page: to Addendum No.:		Report date: 28.06.93 Number: G70 Study period (years): 1989 - 1991												
Number of tumours in all animals which were evaluated (without consideration of the causes and relevance)					Frequency according to dose and sex (n)									
					(0) Contr. A		(4) Contr. B		(1)		(2)		(3)	
Biometrical evaluation yes <x> no <>					m	f	m	f	m	f	m	f	m	f
Number of animals evaluated														
Organ	Identification of the tumour													
BRAIN	OLIGODENDROGLIOMA				1	-	-	-	-	-	-	-	-	-
	NEOPLASM (NOS)				-	-	-	-	-	-	-	-	-	1
LUNGS	ADENOMA				22	9	11	8	12	13	8	6	13	6
	CARCINOMA				1	2	-	-	2	-	1	-	-	-
LIVER	ADENOMA/HEPATOC.				5	-	7	1	2	-	-	-	2	-
	HEMANGIOSARCOMA				3	1	4	-	2	1	1	-	1	-
	CARCINOMA/HEPATOC.				-	-	-	-	1	-	1	-	-	-
TONGUE	CARCINOMA/SQUAMOUS				1	-	-	-	-	-	-	-	-	-
DUODENUM	SARCOMA/OSSIFYING				-	-	-	-	-	-	1	-	-	-
TESTES	LEYDIG CELL TUMOR				4	-	3	-	2	-	-	-	5	-
PROSTATE	ADENOMA				1	-	-	-	-	-	-	-	-	-
SEMINAL														
VESICLES	LEIOMYOMA				1	-	-	-	-	-	-	-	-	-
OVARIES	LUTEOMA				-	-	-	-	-	-	-	-	-	1
	ADENOMA/TUBULAR				-	1	-	2	-	1	-	2	-	-
	TUMOR/GRANULOSA CELL				-	1	-	4	-	2	-	5	-	3
	LIPOMA				-	1	-	-	-	-	-	-	-	-

* P < 0.05 ** P < 0.01

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BOEHRINGER INGELHEIM KG Name of finished product		TABULATED STUDY REPORT		U93-0589									
Name of active ingredient Pramipexole (SND 919 CL 2 Y)		ref. to III.E.210											
		3/6 Page Number											
ONCOGENIC/CARCINOGENIC POTENTIAL Tumour data													
Ref. to document.: Volume: Page: to Addendum No.: Report date: 28.06.93 Number: G70 Study period (years): 1989 - 1991													
Number of tumours in all animals which were evaluated (without consideration of the causes and relevance)				Frequency according to dose and sex (n)									
				(0) Contr. A		(4) Contr. B		(1)		(2)		(3)	
Biometrical evaluation yes < x > no < >				m	f	m	f	m	f	m	f	m	f
Number of animals evaluated													
Organ	Identification of the tumour												
UTERUS	POLYP/STROMAL			-	2	-	-	1	-	3	-	3	
	LEIOMYOMA			-	4	-	1	-	2	-	2	-	2
	FIBROMA			-	-	-	-	-	-	-	-	-	1
	GRANULAR CELL TUMOR (m)			-	-	-	-	-	-	-	-	-	1
	LEIOMYOSARCOMA m			-	-	-	-	1	-	-	-	-	1
	ADENOCARCINOMA m			-	-	-	-	1	-	-	-	-	-
	SARCOMA/STROMAL CELL m			-	2	-	1	-	-	-	2	-	1
VAGINA	FIBROMA			-	1	-	-	-	-	-	-	-	-
URINARY													
BLADDER	CARCINOMA/TRANSIT. CELL			-	-	-	-	-	-	-	-	-	1
PITUITARY													
GLAND	ADENOMA/P. DISTALIS			-	-	-	1	1	2	-	3	-	-
THYROID	ADENOMA/FOLLICULAR			-	-	-	1	-	1	-	-	-	-
	CARCINOMA/FOLLICULAR			-	-	-	-	-	-	1	-	-	-
PARA-													
THYROID													
GLANDS	ADENOMA			-	2	-	-	-	-	-	-	-	-

* P < 0.05 ** P < 0.01

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BOEHRINGER INGELHEIM KG Name of finished product		TABULATED STUDY REPORT				U93-0589								
Name of active ingredient Pramipexole (SND 919 CL 2 Y)		ref. to III.E.210												
		4/6				Page Number								
ONCOGENIC/CARCINOGENIC POTENTIAL Tumour data														
Ref. to document.: Volume: Page: to Addendum No.:		Report date: 28.06.93 Number: G70 Study period (years): 1989 - 1991												
Number of tumours in all animals which were evaluated (without consideration of the causes and relevance)					Frequency according to dose and sex (n)									
					(0) Contr. A		(4) Contr. B		(1)		(2)		(3)	
Biometrical evaluation yes < X > no < >					m	f	m	f	m	f	m	f	m	f
Number of animals evaluated														
Organ	Identification of the tumour													
ADRENAL														
CORTEX	ADENOMA/A-CELL				-	-	-	-	1	-	-	-	-	1
	ADENOMA/B-CELL				6	-	3	-	3	-	6	-	3	1
	ADENOMA/B-CELL/EXID.** (males)				8	-	5	-	3	-	2	-	-	-
	ADENOMA/Z. FASCICUL.				3	-	-	1	1	-	-	-	-	-
ADRENAL														
MEDULLA	MEDULL. TUMOR/BENIGN				1	-	-	-	-	-	-	-	-	-
	MEDULL. TUMOR/MALIGN.				-	-	-	1	-	-	-	-	-	-
MESENT.														
LYMPH NODE	HEMANGIOMA				-	-	-	1	-	-	-	-	-	-
SYSTEMIC														
NEOPLASMS	THYMIC LYMPHOMA* (females)				3	18	4	16	4	13	4	8	4	7
	NONSPECIF. LYMPHOMA				1	-	1	1	1	-	-	-	-	-
	NON-THYMIC LYMPHOMA				3	5	4	3	6	3	3	3	4	1
	MAST CELL TUMOR				1	-	-	-	-	-	2	-	-	1
	BONE MARROW LYMPHOMA				-	-	-	1	-	-	-	-	-	-
	HISTIOCYTIC SARCOMA				-	1	-	4	4	2	-	2	-	1
	MYELOID LEUKEMIA				-	1	-	-	-	-	-	-	-	-

* P < 0.05, ** P < 0.01 (=negative trend) -

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Tab. C.S.a.6. (cont.)

BOEHRINGER INGELHEIM KG Name of finished product		TABULATED STUDY REPORT				U93-0589								
Name of active ingredient Pramipexole (SND 919 CL 2 Y)		ref. to III.E.210												
		5/6				Page Number								
ONCOGENIC/CARCINOGENIC POTENTIAL Tumour data														
Ref. to document.: Volume: Page: to Addendum No.:		Report date: 28.06.93 Number: G70 Study period (years): 1989 - 1991												
Number of tumours in all animals which were evaluated (without consideration of the causes and relevance)					Frequency according to dose and sex (n)									
					(0) Contr. A		(4) Contr. B		(1)		(2)		(3)	
Biometrical evaluation yes <x> no <>					m	f	m	f	m	f	m	f	m	f
Number of animals evaluated														
Organ	Identification of the tumour													
HARDERIAN														
GLANDS	ADENOMA				2	1	2	2	2	3	-	-	-	-
	ADENOCARCINOMA				-	-	-	-	-	1	-	-	-	-
SPLEEN	HEMANGIOSARCOMA				-	-	-	-	-	1	-	-	-	-
SKIN	LIPOMA				-	-	1	-	-	-	-	-	-	-
	HEMANGIOMA				-	-	-	-	-	1	-	-	-	-
	HEMANGIOSARCOMA				1	-	-	-	-	1	-	-	-	-
	CARCINOMA/BASAL CELL				-	-	-	-	-	1	-	-	-	-
	CARCINOMA/SQUAMOUS				-	-	-	-	-	1	-	-	-	-
	SARCOMA/OSSIFYING				-	1	-	-	-	-	-	-	-	-
	LEIOMYOSARCOMA				-	1	-	-	-	1	1	-	-	1
	SCHWANNOMA				-	-	-	1	-	-	-	-	-	-
	SARCOMA/UNDIFFERENT.				-	-	-	-	-	-	1	-	-	-
BODY														
CAVITIES	LIPOMA				-	-	-	-	-	-	1	-	-	-
	SARCOMA/OSSIFYING				1	-	-	-	-	-	-	-	1	-
	SARCOMA/UNDIFFERENT.				-	-	-	1	-	-	-	-	-	1

* P < 0.05 ** P < 0.01

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